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REFLECTANCE INDICES WITH PRECISION AND ACCURACY IN PREDICTING COTTON LEAF NITROGEN CONCENTRATION

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Abstract

Diagnostic methods assaying leaf optical properties can aid rapid site-specific screening of crop nitrogen status. A set of calibration curves relating many 1.5-nm band reflectance ratios to cotton (*Gossypium hirsutum* L.) leaf N concentration was established from plants grown in sunlit growth chambers and at a range of nitrogen levels. Predicted and actual concentrations were compared by regression for a validation set of field-grown leaf samples from diverse genotypes. Only those ratios that combined a red-edge measure (700 or 716 nm) with a waveband of high reflectance in the very near infrared region (755–920 and 1000 nm) provided good precision (correlation) and accuracy (one-to-one relationship between predicted to actual values). Other indices that included a chlorophyll-based reflectance feature also had good precision but were less accurate than those obtained from the red-edge/very-near-infrared reflectance ratios.

NITROGEN FERTILITY often limits the yield of cotton (Gerik et al., 1998) and other crops. Site-specific applications and massive germplasm screening are novel technologies that can be used to address nitrogen fertility effects on yield. These technologies require, however, that the current methods for determining the nitrogen nutritional status of small neighborhoods of plants be supplemented with methods that are increasingly rapid and have the capability of examining numerous sites in a single field. Diagnostic methods that take advantage of the optical properties of leaves possibly provide apt supplementary indices because they can be used in translation of spectroradiometric data into site-specific measures of nitrogen status.

Specificity for N concentration when constructing an index based on leaf reflectance requires the selection of one, several, or multiple bands of response. Many of the radiation-based indices for leaf N concentration involve a measure of chlorophyll concentration (Tsay et al., 1982; Nelson et al., 1986) because chlorophyll concentrations are typically less in nitrogen-limited leaves (Mengel and Kirkby, 1982). Methods are needed to establish specificity response surfaces that are appropriate for optimizing radiation-based indices for simultaneous determination of concentrations of multiple compounds.

In a previous study, the reflectance spectral regions of soybean leaves sensitive to nitrogen deficiency were determined by dividing the spectrum obtained from leaves grown under optimal conditions by the spectrum from leaves of plants grown at medium nitrogen levels (Chappelle et al., 1992). Even though there was an overall increase in reflectance with nitrogen deprivation, several broad regions of the reflectance spectrum increased relatively more. These included the region from 500 to 630 nm (green and yellow reflectance), the region from 670 to 720 nm (*red edge*), and to a lesser extent from 400 to 460 nm (blue). The red edge is a transition region of the reflectance spectrum between high absorbance by pigments in the visible region and high reflectance by leaf structural features in the very near infrared region (Curran et al., 1990).

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Abbreviations: CDT, Central Daylight Savings Time; SPAR, Soil Plant Atmosphere Research.

A similar type of analysis was performed for leaves of plants subjected to diverse stresses (Carter, 1993, 1994). The same two spectral regions were identified as in Chappelle et al. (1992). These regions had similar maximal sensitivities of 535 to 640 nm and 685 to 700 nm. The use of ratios between reflectance radiance values of stress-sensitive bands and stress-insensitive bands as a way to correct for variations in irradiance, leaf orientation, irradiance angles, and shading was also proposed. Carter (1994) constructed indices to include one stress-sensitive spectral band and one insensitive band. The ratios that changed significantly and consistently between nonstressed and various stressed leaves were considered the best general-stress indices. These included, in order of strength of indication, the ratios of reflectances at 420 to 695 nm, 695 to 760 nm, 710 to 760 nm, and 605 to 760 nm.

Derivative spectra can also be used to identify spectral regions that are sensitive to a particular stress. When the derivative spectra of control and nitrogen-limited leaves were compared, the regions of the reflectance spectrum similar to those identified in the above studies [approximately 500–550 nm (green reflectance) and 685–715 nm (the red edge)] were most sensitive to nitrogen limitation (Peñuelas et al., 1994).

Grain yield is often sensitive to nitrogen deprivation, and the yield from nitrogen-fertility experimental plots has been used as an indicator of nitrogen deficiency (Blackmer et al., 1996). The same two regions of sensitivity to nitrogen deficiency were identified. These authors used grain yield as an indicator of nitrogen status of various plots and demonstrated that reflectance at 550 nm, 710 nm, and the ratio of reflectance of the 550 to 600 nm region with the 800 to 900 nm region provided good precision.

The results from the above studies indicate that there are spectral patterns that correlate with known nitrogen deficiency but that the sensitive regions might not differ from those of other stresses. To determine the potential for distinguishing among various deficiencies based on their reflectance spectral pattern, the spectral properties of leaves with deficiencies in iron, sulfur, magnesium, and manganese were examined (Masoni et al., 1996). The spectral regions of most sensitivity to any of these mineral deficiencies were similar to those sensitive to nitrogen deficiency (Chappelle et al., 1992; Peñuelas et al., 1994; Blackmer et al., 1996) and general stress (Carter, 1994). This indicates a lack of selectivity by reflective indices for nitrogen status and consequently a decrease of accuracy in predicting nitrogen status.

The goal of this study was to establish a method for determining the nature of indices for estimating cotton leaf N concentration that provide accuracy, in addition to precision, when these rapid, but indirect, optical detection methods are used. Precision and accuracy are dual criteria for estimation. The specific objectives of this study were to: (i) develop reflectance indices of leaf N concentration, using information in the 350 to 1050 nm portion of the spectrum; (ii) identify the regions of the spectrum that have a relatively strong positive influence on precision and accuracy of reflectance indices of leaf N concentration including the establishment of precision and accuracy response surfaces for the spectrum; and (iii) define one or more indices, constructed as ratios of narrow-band reflectances, with good accuracy and precision (i.e., the relationship between predicted leaf N concentration and the actual concentration has a one to one correspondence and a high correlation value).

Materials and Methods

General Procedure

Reflected radiance was collected from cotton leaves having a range of leaf N concentrations. Relationships derived between leaf N and various reflectance indices were tested on several field-grown cotton varieties for potential as predictors of leaf N in field-grown plants. The derived indices were compared to judge the precision and accuracy of various regions of the reflectance spectrum.

Soil-Plant-Atmospheric-Research (SPAR) units, the closed-environment plant growth chambers used for this study were described in detail by Phene et al. (1978), Acock et al. (1985), and Reddy et al. (1992). The SPAR units are located outside and provide control of temperature and CO_2 concentration at predetermined set points for plant growth studies in natural solar radiation regimens. Each SPAR unit consists of a steel soil bin (1 m tall by 2 m long by 0.5 m wide), a poly(methylmethacrylic) chamber (2.5 m tall by 2.0 m long by 1.5 m wide) to accommodate aerial plant parts, a heating and cooling system, and an environmental monitoring and control system. Poly(methylmethacrylic) is transparent to solar radiation between 280 nm and 1100 nm except for a strong absorbance region centered at 363 nm with a full width at half maximum of 50 nm (1999, unpublished results).

Seeds of cotton cv. Nucotn33B were planted in four SPAR units during June 1998 in three rows of five plants per row, 15 plants m^{-2}. The chamber soil bins were filled with fine sand. Throughout the experiment, the temperature in the chambers was maintained at 30/22°C, day/night. The plants were grown in three carbon dioxide treatments: 180, 360 (ambient), and 720 $\mu L\ L^{-1}$. A half-strength Hoagland's nutrient solution was applied three times daily to each growth chamber via a drip irrigation system. At initial flowering, 53 d after emergence, the units were leached with water, and one unit in each of the carbon dioxide treatments was supplied nutrients with a modified osmotically balanced Hoagland's solution containing no nitrogen while the other chambers were supplied with the complete Hoagland's solution. The total water each day was twice the previous day's evaporation. Excess water was allowed to drain from small openings at the bottom of the soil bin. These are near-optimal growth conditions for cotton (Reddy et al., 1992; Reddy et al., 1996).

The uppermost fully expanded leaves collected from plants grown in the SPAR units were used when constructing calibration curves relating reflectance values and leaf N concentration. Near mid-day at mid-fruiting stage, the reflectance spectra were obtained from three to six leaves in each chamber. The leaves were placed adaxial side up on top of a black polyurethane background that had greater than 95% absorbance of incident radiation throughout the spectral range evaluated in this study. The spectroradiometer used was a GER 1500 (Geophysical & Environmental Research Corp., Millbrook, NY), which had a range of 350 nm to 1050 nm

with a sampling interval of 1.5 nm. The spectra were captured for approximately 2.5-cm-diam areas of a lower lobe. The radiance was collected from a nearly perpendicular angle to the leaf surface while avoiding shadows falling on the leaf. Reference spectra were collected from a white Mylar high reflectance panel in order to ensure that the spectroradiometer performance was consistent throughout the period of spectra collection. Single spectra were obtained for each leaf under clear sky solar radiation within 1 min after clipping. The leaves from each chamber were then pooled, dried at 70°C for 72 h, ground to less than 1 mm^2 in diameter, weighed, and submitted to a service laboratory for determination of total N by routine procedures (Nelson and Sommers, 1972).

Field Plots

Field studies were used for validation. Genotypic variation was introduced through the use of six cotton cultivars that were planted in May 1997 in 1-m rows oriented north to south. The genotypes used in the study included four that are obsolete: Lone Star (released in 1905), Half-and-Half (1910), Deltatype Webber (1922), and Coker Wilds (1929). Two modern genotypes, Suregrow 125, and Stoneville 474, were also included. We anticipated that the genotypes would vary in a number of traits that could potentially influence leaf optical properties, such as wax composition and hair distribution. The genotypes provided variation independent of that due to imposition of N deficiency in the SPAR-unit study and therefore appropriate for establishing indices specific for N in the presence of variable interference and matrix effects. Standard agricultural practices were followed for weed and insect control of all plots, which were located on the Mississippi State University farm near Starkville, MS.

Ten uppermost fully expanded leaves from each of the cultivars were used for the validation study. The reflected radiance and leaf N concentration were collected from the same individual leaves. The data from all field-grown leaves were pooled for statistical analysis. During late boll-opening stage, the reflectance spectra were obtained under clear skies from the same intact leaves at two times of the day, 1200 to 1300 and 1500 to 1600 h CDT, by means of procedures similar to those used for the calibration leaves with two exceptions. Leaves were oriented with adaxial side to the sun and without any background material directly underneath. The radiance was again collected from an angle nearly perpendicular to the leaf surface while avoiding shadows.

An empirically based prescreen was performed on the field-grown leaf samples to minimize variation due to specular reflectance. Specular reflectance, or glare, alters reflected radiance but has no physiological interpretation, as opposed to diffuse reflectance (Grant, 1987), and so can be eliminated from analyses of spectral change due to variation in leaf composition. Arbitrary ratios of reflectance bands were compared between the two sampling times without regard to N concentration, and the 20% of leaves that produced the largest number of outliers in the time of day comparison were eliminated from the statistical comparison of leaf N concentration and reflectance indices (McDonald, 1981; Wilcox, 1997). The rationale for this approach is (i) leaf pigments are the major absorbing component in the examined interval of the radiation spectrum, but would not be expected to change in concentration very much in a few hours; (ii) another cause of diurnal change in the leaf reflectance spectra is diurnal change in the incident radiation, but this would be consistent among leaves; (iii) physiological changes primarily affect certain regions of the reflectance spectrum, but the use of an arbitrary ratios procedure emphasizes the ability to detect change throughout the whole spectrum; and (iv) the final major contributor to diurnal differences in leaf spectra is specular reflectance, but this is additive with diffuse reflectance and would lead to altered waveband ratios in those leaves for which it was present due to poor leaf or spectroradiometer positioning at one of the two sampling times—these leaves would show up as outliers in the prescreen. The N concentrations of the leaves that were not eliminated during the prescreening process were compared with the predicted leaf N concentrations. The predicted N values were derived from the reflectance spectra obtained at the 1200 to 1300 h CDT sampling time, as described in the following section. The next day at 1100 h CDT, the same leaf blades were harvested directly into liquid nitrogen. At a later date, the blades were dried at 77°C for 48 h, ground to less than 1-mm^2 diameter, and submitted to a service laboratory for determination of total N by routine procedures (Nelson and Sommers, 1972).

Mathematical Manipulations and Statistical Analysis

Twenty 1.5-nm-wide bands of the reflectance spectra were selected by visual inspection to represent the spectral features (360, 416, 460, 495, 516, 525, 555, 590, 640, 685, 700, 716, 755, 808, 820, 843, 920, 953, 965, and 1000 nm) (Fig. 1). The twenty bands were enough to represent the spectral information, yet few enough to allow the data analyses to be performed easily with commonly available computer hardware and software. The reflectance spectrum was mapped into a more convenient mathematical space that retained the information concerning the spectral shape. The new space consisted of all possible ratios between reflected radiance values of the twenty bands. This mapping resulted in 190 ratios, or indices (i.e., the reflected radiance at the band centered at 360 nm divided by the reflected radiance at the band centered at 416 nm, radiance at 360 nm divided by radiance at 460 nm,..., radiance at 965 nm divided by radiance at 1000 nm).

The univariate least squares linear regressions between the indices (herein, the set of reflectance ratios at two wavebands) and leaf N concentration were obtained. The regressions were used to make multiple estimates of leaf N concentration of the field-grown leaf samples based on their reflectance measurements.

The predicted and actual N concentrations of the field-

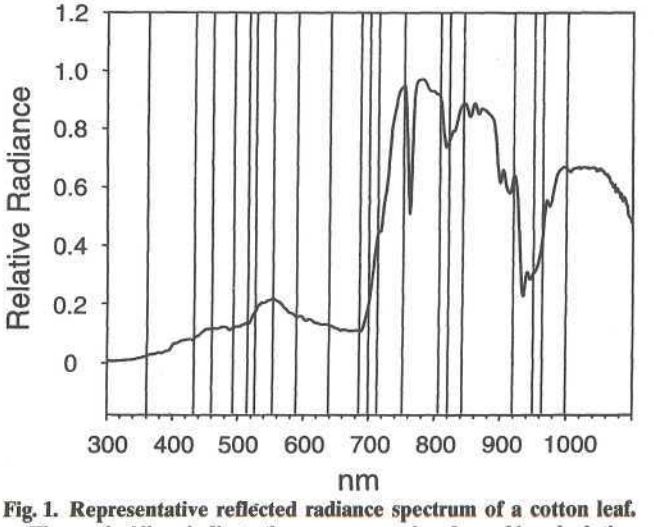

Fig. 1. Representative reflected radiance spectrum of a cotton leaf. The vertical lines indicate the narrow wavebands used in calculating multiple ratios of radiance values.

grown leaf samples were compared by univariate least squares regression. The regressions were examined for precision (a correlation value close to 1.0 would indicate high precision) and accuracy (slope close to 1.0 when the intercept is zero would indicate high accuracy) (Massart et al., 1988). Univariate least squares regressions were also performed when the intercept was not forced to be zero.

Clusters of ratios were identified and characterized that contributed to good precision or accuracy or both. Precision and accuracy response surfaces also result from this method.

Results and Discussion

The correlations of linear fit (r) of about half (94 of 190) of the indices with leaf nitrogen had absolute values of 0.8 or greater, with a maximum greater than 0.96. The widespread occurrence of strong correlations of leaf N concentration with reflectance ratios obtained from many parts of the spectrum indicates broad changes in the reflectance spectra were somewhat consistent in response to nitrogen deficiency. These broad changes make it very difficult to determine visually which portions of the spectrum are most sensitive to change in leaf N concentration and so justify the use of mathematical analysis of the spectral changes. Furthermore, the best indices are not necessarily those with the highest correlation in a particular study, but are rather those that perform consistently well in diverse situations. Regardless of the extent of correlation, all reflectance ratios were regressed against leaf N concentration to obtain calibration curves. The averages of the leaf N concentrations used for the calibration curves ranged from 19.0 to 40.5 g kg^{-1} dry wt.

All of the 190 generated calibration curves were retained for testing the prediction equations. This resulted in 190 predicted and 1 actual N concentration for each field leaf sample. The leaf N concentrations for the validation samples ranged from 18.2 to 42.7 g kg^{-1} dry wt with >90% falling within the range of the calibration data set. Some of the calibration curves using particular indices predicted leaf N concentration poorly, but others predicted leaf N concentration well. No single waveband always led to good indices and every waveband contributed to at least one index good for precision or accuracy, but some regions of the spectrum were more likely to contribute to good waveband-ratio indices. The primary interests were to identify the independent response patterns by regions of the spectrum relative to the precision and accuracy (Fig. 2) and to identify regions that combine well. The indices with precision or accuracy above a certain level were clustered on the basis of the patterns observed in Fig. 2 according to the following rules: (i) If a ratio of the radiances at two wavelengths provided good precision or accuracy, then the box that is the intersection of the two wavelengths is hatched or solid, and (ii) If a hatched or solid box was no more than two boxes (i.e., two spectral features) removed in a horizontal or vertical direction from another hatched or solid box then the two boxes were clustered. The following discussion will focus on the clusters rather than individual indices.

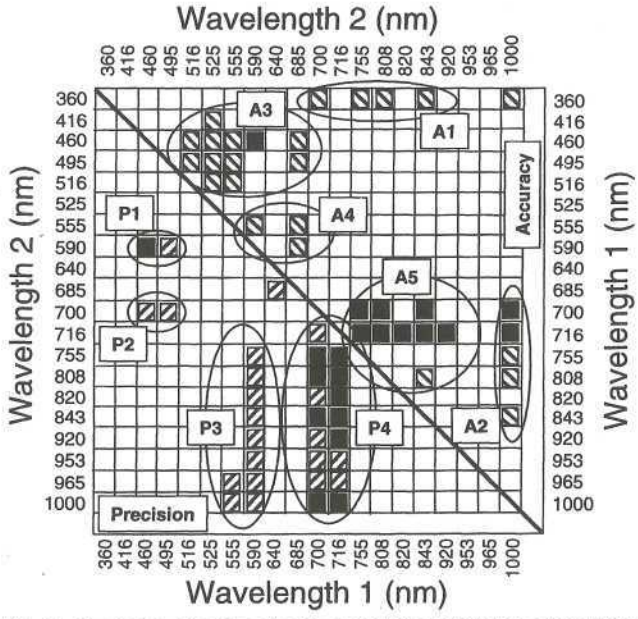

Fig. 2. Clustering of ratios of radiances that provided good accuracy (linear regression of predicted and actual leaf total N concentrations of test samples had a slope between 0.88 and 1.12 when intercept = zero) (upper right triangular panel), good precision (correlation between predicted and actual concentrations ≥ 0.65) (lower left triangular panel), or both (solid squares). The solid diagonal line is the panel separator.

Thirty-one of the correlations of linear relationship between predicted and actual leaf N concentration were equal to or greater than 0.65 (the maximum was 0.74). The 0.65 value was chosen to retain the best performing 15 to 20% of predictors. These fell into four clusters (Fig. 2, lower left triangular panel): P1—ratios using 460 or 495 nm combined with 590 nm (these wavebands are on either side of the reflectance peak in the green, thus these are blue to yellow ratios), P2—ratios using 460 or 495 nm combined with 700 nm (associated with the red edge), P3—ratios using 555 (green reflectance) or 590 nm (a band of low reflectance associated with absorbance by pigments) combined with various wavebands of the very near infrared reflectance plateau (755 to 1000 nm), and P4—ratios using 700 or 716 nm (measures of the red edge) combined with various wavebands of the very near infrared reflectance plateau. All of the ratios that provided good precision through the associated calibration curve contained at least one waveband that was in a region sensitive to nitrogen limitation or general stress (Chappelle et al., 1992; Carter, 1994; Peñuelas et al., 1994; Blackmer et al., 1996; Masoni et al., 1996). The other waveband of each ratio was not in one of the two most sensitive regions. This would help to normalize the index with respect to leaf properties (i.e., these wavebands are good choices for reference wavebands).

Univariate least squares linear regression when the intercept is not forced to be zero allows accuracy to be examined in detail. A change in slope when the intercept is near zero indicates proportional error with little constant error. In contrast, an intercept distant from zero with a slope near one indicates a constant error with

little proportional error. Proportional error occurs when a certain percentage of the analyte (leaf N) is not detected, whereas constant error is often due to the detection of another undesired compound (Massart et al., 1988). These separate effects on accuracy can also be present in combination. There was a strong relationship in this study ($r = -0.96$) between the slope and the intercept for the calibration curves selected for high precision—the lesser the slope, the greater the intercept (Fig. 3). In other words, many of the indices that were precise were not accurate because a percentage of the N was not detected and because other compounds were contributing to the signal. The practical result from this relationship is that we were not able to separately examine the contributions of spectral regions to proportional and systematic errors. The following results describe accuracy by the closeness of the slope to 1.0 when the intercept is forced to zero. In this analysis, the proportional and systematic errors acted together to alter the slope.

Thirty-four of the linear regressions between predicted N and actual N had a slope between 0.88 and 1.12 (i.e., fairly good accuracy). The 0.88 to 1.12 range was chosen to retain the 15 to 20% best-performing predictors. These fell into five clusters (Fig. 2, upper right triangular panel): A1—360 nm (near ultraviolet) combined with bands of the very near infrared from 700 to 1000 nm; A2—1000 nm combined with bands of the nearer part of the very near infrared from 700 to 843 nm; A3—ratios using 460 or 495 nm (blue) and to a lesser extent 416 nm (violet) and 516 nm (blue-green) combined with wavebands of the green reflectance (525 and 555 nm) and to a lesser extent with the blue-green (516 nm), yellow (590 nm) and red (685 nm); A4—green (555 nm) or yellow (595 nm) combined with the yellow or red (685 nm); and A5—the near part of the very near infrared (especially the red edge, 700 and 716 nm) combined with the somewhat more distant portion of the very near infrared reflectance plateau (755 to 920 nm). With the exception of the A1 cluster, the ratios that provide good accuracy are those combining neighboring features of the spectrum. One of the wavebands in this situation is possibly tending to function as a baseline.

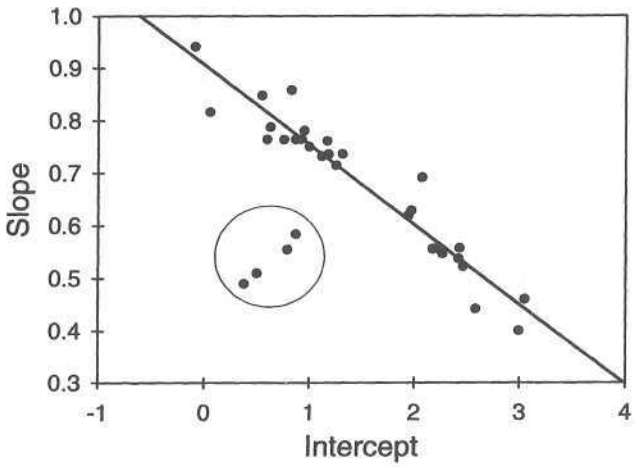

Fig. 3. Scatter plot of slope and intercept for calibration curves that displayed good precision ($r \geq 0.65$). Equation for regression line: *slope* = 0.91 − 0.15(*intercept*). The group of four encircled data points was removed from the regression analysis. These four data points differ from the others because they resulted from calibration curves based on a reflectance index that included a contribution from the blue region of the reflectance spectrum.

Only those ratios that combined a red-edge measure (700 or 716 nm) with a waveband of high reflectance in the very near infrared region (755–920 and 1000 nm) provided both good precision (correlation) and accuracy (slope near 1 when intercept at 0). For example, the calibration curve associated with the 716 nm to 808 nm reflectance ratio provided a good estimator of actual N concentration with a slope of 0.97 and $r = 0.74$. The calibration curve associated with the 716 to 1000 nm reflectance ratio provided a slope of 0.94, an intercept of –0.09 and $r = 0.71$ when the slope was not forced to zero, and did not significantly differ from a line with slope of 1 and intercept of zero. The very near infrared region is possibly acting both to: (i) normalize the index with respect to leaf properties and so increase precision, and (ii) provide a baseline for red-edge wavebands and so increase accuracy. The indices of clusters P1 and P2 proportionately overestimated leaf N concentration (slopes of 1.12–1.57), while those of P3 proportionately underestimated leaf N concentration (slopes of 0.66 to 0.77). The position of the red edge was shown to correlate well with leaf N concentrations ($r = 0.62$) (Peñuelas et al., 1994). In addition, the ratio of reflectance at 710 nm (red edge) to that of the 800 to 900 nm band (very near infrared region) can correlate quite well with corn grain yield (a measure of N status under some agronomic conditions) (r^2 of 0.68–0.99) (Blackmer et al., 1996). Furthermore, the ratio of reflectance at 695 or 710 nm (red edge) to 760 or 800 nm (very near infrared region) was an excellent general stress indicator (Carter, 1994). The fairly good correlation of members of the P4–A5 cluster to leaf N concentration was expected, based on previous literature reports. The good accuracy obtained by members of the cluster indicates that specificity was also achieved.

The precision provided by other indices that include a chlorophyll-based reflectance feature (especially P3; mean $r = 0.67$) is significantly less ($P = 0.0001$) than that of the indices of the best-performing cluster (P4; mean $r = 0.72$). The predicted values of these chlorophyll-based indices (for P3: mean slope = 0.73), furthermore, are proportionately lower ($P = 0.0001$) than those of the best-performing cluster (for P4: mean slope = 0.90). This indicates a lowered sensitivity of some chlorophyll-based indices to leaf total N in field-grown leaves relative to SPAR-unit grown leaves, presumably due to the influence of other factors associated with chlorophyll concentration (Carter, 1994; Masoni et al., 1996; Sunderman et al., 1997), and possibly lack of the very near infrared region contribution to normalization and baselining.

We have presented an improved method for determining the reflectance spectral regions providing good precision and accuracy for predicting cotton leaf

nitrogen concentration. Indices that are both precise and accurate are needed to achieve selectivity when used in combination to simultaneously predict the concentrations of multiple compounds. The method should be readily applicable to analysis of other species and other responses.

The advantage of the method is due to examination of the predictive value of all possible ratios between reflected radiance values of a number of pre-selected bands. By using this systematic approach, we can rapidly evaluate which regions of the spectrum contribute more to precision or accuracy or both relative to other regions. The method contributes towards developing optimal procedures for interpreting the physiological status of leaves through analysis of their reflectance spectra.

We recommend the inclusion of ratios combining wavebands of the red edge and the very near infrared region as choices during evaluation of simple reflectance indices for leaf total nitrogen. The combination of red edge wavelengths with very near infrared wavelengths provides good precision and accuracy in predicting leaf N concentration compared to other ratios utilizing green reflectance or yellow-orange absorbance features.

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